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LLT USES THEREOF IN IMMUNE SYSTEM MODULATION

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FIELD OF THE INVENTION

The present invention relates generally to the field of specific cell surface receptors, and in particular characteristic peptides of these receptors. Even more particularly, it concerns cell surface receptor peptides on cell types important in governing immune system response, such as these cells known as natural killer (NK) cells. The present invention also relates to methods of treating immune function disorders. In particular embodiments, these methods apply various of the cell surface receptor peptides identified by the present inventors in therapies directed to enhance immune cell function, and consequently provide a treatment for immune system related pathologies.

BACKGROUND OF THE INVENTION

The molecular basis of target cell recognition by NK cells is poorly understood. Unlike T and B cells, NK cells do not rearrange DNA to generate diversity. Therefore, one could predict that NK cells might express several receptors to recognize various targets or utilize some other mechanism to generate diversity. In fact, over the last few years a number of receptors have been identified on NK cells (Lanier 1998). However, all the function of NK cells could not be accounted by the known receptors.

The C-type carbohydrate recognition domain (CRD) is the common feature among Ca^{2+}-dependent animal lectins and structurally related proteins. A subset of the C-type lectin family found on natural killer (NK) cells contains domains homologous to other C-type lectin domains, but whether they mediate interactions through carbohydrate or protein binding remains unresolved (Weis et al. 1998). NK cell receptors with lectin-like domains are encoded in the NK gene complex on Chromosome (Chr) 6 in the mouse and Chr 12 in the human. (Brown et al. 1997; Yabe et al. 1993; Yokoyama and Seaman 1993).

The majority of NK cell receptors encoded by the NK gene complex belong to groups of highly related genes such as the NKR-P1, Ly-49, and NKG2 families. The Ly-49 and NKG2 families contain members that are mostly inhibitory, but have a few members that transduce activation signals (Lanier 1998; Long and Wagtmann 1997; Yokoyama and Seaman 1993). The NKR-P1 receptors have been observed to act as activating receptors in rodents. Cross-linking of the human NKR-P1 homologue with antibody leads to inconsistent results (Lanier 1998). CD94 is a type II receptor express on most NK cells and was originally implicated as an inhibitory receptor (Change et al. 1995; Long and Wagtmann 1997). Subsequently, it was discovered to form a heterodimer with members of the NKG2 family (Lazetic et al. 1996).

CD69 and AICL (activation-induced C-type lectin) are two structurally similar receptors localized to the NK gene complex, but have interesting differences from the other genes located there. As opposed to the other type II receptors in the NK gene complex, which are restricted to NK cells and a subset of T cells, CD69 and AICL are expressed in most cells of hematopoetic origin (Hamann et al. 1997; Lanier 1998; Long and Wagtmann 1997; Testi et al. 1994). The function of AICL is not know, but CD69 cross-linking leads to the activation of NK cells, T cells, B cells, monocytes, granulocytes, and platelets (Testi et al. 1994). In addition, these genes appear to have single rather than multiple isoforms.

Despite advances that have been made in the area of immune cell function, a need continues to exist in the medical arts for improved techniques for antibody tumor growth and other cancer forms such as leukemia. In addition, insofar as bone-marrow grating has been used, significant immune rejection difficulties preclude the use of these techniques for improving and/or replacing immune function in animals, and most importantly, preventing host rejection.

SUMMARY OF THE INVENTION

The present invention, in a general and overall sense, concerns a novel cell surface receptor localized on natural killer (NK) cells.

In the present invention, the molecular cloning, characterization, and expression pattern of a new lectin-like transcript predominantly expressed on human NK cells is defined. In

particular, the molecular characterization of LLT1 and LLT2 is provided in the present invention.

This study describes the cloning and molecular characterization of a new member of the human NK gene complex. The conserved C-typed CRD found in NK cells receptors localized to the NK gene complex allowed identification of related sequences in the EST database. LLT1 is localized to the human NK gene complex on Chr 12, close to CD69. The predicted peptide of LLT1 (FIG. 1A) bears similarity to CD69 and AICL (Hamann et al. 1993, 1997; Lopez-Cabrera et al. 1993; Ziegler et al. 1993). The function of CD69 has been extensively characterized. Cross-linking with anti-CD69 Ab activates the cell-specific functions of lymphocytes, granulocytes, monocytes, and platelets (Testi et al. 1994). CD69 is a useful indicator of cell activation and immune arousal. In light of the structural similarity of CD69 to AICL and LLT1, it is likely these new transcripts will demonstrate novel functions.

In some embodiments, the present invention provides a polypeptide of the LLT1 cell receptor. In one aspect, this polypeptide may be described further as comprising a transmembrane domain near the N-terminus of the polypeptide, and as having an overall length of about 185 to about 205 amino acid residues. In particular embodiments, the length of the polypeptide is about 191 amino acid residues and is essentially free of intracellular ITIM motifs, having a molecular weight of between about 25 kilodaltons and about 65 kilodaltons. In some embodiments, the transmembrane domain of the polypeptide has an amino acid sequence of SEQ ID NO:1. In other embodiments, the peptide may be further defined as having a sequence of SEQ ID NO:2. The polypeptide may also be defined as further comprising an intracellular domain of a sequence defined in SEQID NO:3 (30 amino acids).

In some aspects, the polypeptide is defined as further comprising an extracellular domain of about 130 amino acids, wherein said extracellular domain includes at least a first putative N-linked glycosylation site and a second putative N-linked glycosylation site. The glycosylation site is located within said polypeptide at a position defined as an amino acid position 95 to an amino acid position 97 and said second putative N-linked glycosylation site is located within said polypeptide at a position defined as an amino acid position 147 to an amino acid position 149.

The polypeptide of the invention may be formulated as a pharmaceutically acceptable preparation, and particularly as a pharmaceutically acceptable preparation suitable for injection.

In some aspects, the polypeptide may be further defined as having a length of about 185 amino acid residues to about 205 amino acid residues. In preferred aspects, the polypeptide may be defined as having an amino acid length of about 191 amino acid residues.

In another aspect, the invention may be defined as a natural killer cell receptor polypeptide comprising the polypeptide as described above. For example, the natural killer cell receptor polypeptide is defined as comprising the sequence of Fig. 1A.

In another embodiment the polypeptide is defined as a clone Y9A2 having a transcript LLT-1, comprising a sequence as defined in Fig. 3A.

The invention also provides a method for inhibiting tumor cell growth. In some embodiments, the methods comprise the steps of:

administering a tumor-cell inhibiting amount of a pharmaceutically acceptable preparation comprising the polypeptide of claim 1 in a tumor-inhibiting amount; and

inhibiting tumor cell growth. According to at least some aspects of the method, the pharmaceutically acceptable preparation is further defined as a physiologically acceptable injectable preparation.

The invention also provides for antibodies having binding affinity for the LLT1 receptor peptide. This antibody may be defined as without binding affinity for a cell surface receptor peptide LLT2. The antibody of the present invention may be defined as either a monoclonal antibody or as a polyclonal antibody. Methods for preparing each are know to those of skill in the art as standard antibody generating techniques and as further defined in the following examples.

The invention also defines a method for reducing natural killer cell mediated rejection of a bone-marrow graft, which comprises:

obtaining bone marrow from a patent to be a receptor of a bone marrow graft; and

treating said bone marrow to a pharmacologically active preparation of the natural killer cell receptor polypeptide of claim 10 to provide a treated bone marrow preparation;

administering a pharmacologically active preparation of the treated bone marrow preparation to said patient.

The invention also provides various cDNA molecules for the LLT1 and the LLT2 receptor peptides. In one such embodiment, the cDNA has a nucleic acid sequence encoding a human lectin-like transcript (LLT1) as defined in Fig. 1A.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG 1 (1A, 1B) Analysis of the cDNA sequence of LLT1. Fig. 1A The nucleotide sequence and predicted translation of LLT1. The transmembrane domain is *underlined*. Glycosylation sites in the extracellular domain are *boxed*. Fig. 1B Hydrophilicity plot of the LLT1 putative peptide sequence determined by the Kyte-Doolittle method.

FIG 2 Pileup of LLT1 and other NK cell C-type lectin superfamily receptors. Conserved residues are *shaded* and putative glycosylation sites in the extracellular domains are *boxed*.

FIG 3 (3A, 3B) RNA blot analysis of LLT1 transcripts hybridized with ^{32}P-labeled, full-length LLT1 cDNA. Fig. 3A Total RNA (20 μg) isolated from the YAC-1, HL-60, DB, Jurkat, and YT tumor cell lines was electrophoresed in a formaldehyde agarose gel, blotted, and probed. In addition, samples were included from the PBMC and a LAK culture from a healthy donor. Fig. 3B Northern blot of poly (A)$^+$ RNA from spleen, lymph node, thymus, peripheral blood leukocytes, bone marrow, and fetal liver tissues. Both membranes were stripped and hybridized with a β-actin probe. The position of 28 S and 18 S rRNA and the sized of RNA molecular standards are shown at the *left* and *right* of panels A and B, respectively.

FIG 4 Genomic DNA blot analysis of human genomic DNA (20 μg) from liver were digested with the restriction enzymes *Bam*HI, *Eco*RI, *Hind*III, and *Xba*I, electrophoresed in 0.8% agarose, blotted, and hybridized with a ^{32}P-labeled, full-length LLT1 cDNA. Sizes of DNA standards are shown at the left.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Following long-standing patent law convention, the terms 'a" and "an" mean "one or more" when used in this application, including the claims.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1 - Expressed Sequence Tag Database Search and cDNA Library Screening

The expressed sequence tag (EST) database at GenBank (http://www.ncbi.nlm.nih.gov) was searched with the TblastN program vs. a consensus sequence of human (CD69, CD94, and NKG2's) and mouse (Ly-49's) C-type lectin receptors (Boguski et al. 1993, 1995). Several overlapping clones were identified and polymerase chain reaction (PCR) primers were designed to amplify a 350 base pair (bp fragment within the C-type lectin CRD. CDNA from a NK cell library constructed in λ phage by J. Houchins (R & D Systems, Minneapolis, MN, and kindly provided by A. Brooks, NIH, Bethesda, MD) was successfully used as template. PCR cycle conditions were 94° C for 30 s, 50° C annealing temperature for 30 s, and a 72° C extension for 45 s repeated for 30 cycles using Taq DNA polymerase from GIBCO BRL (Grand Island, NY) at

2 mM $MgCl_2$. The same library was then screened with the resulting PCR fragment labeled with α^{32}O d/ctO (Feinberg and Vogelstein 1983; Sambrook et al. 1989). Approximately 5 x 10^5 clones were screened. After three rounds of screening, phage DNA was isolated from positive clones by the method of Lee and Clark (1997). All positively selected clones were sequenced (Automated sequencing facility, Department of Pathology, UT Computer Group, Wisconsin package). One clone (Y9A2) which contained an open reading frame was identified for further study. The transcript was named LLT1 (lectin-like transcript 1) due to sequence similarity to other C-type lectin-like receptors found on NK cells.

EXAMPLE 2 - Cell Culture

Human tumor cell lines Jurkat (T cell), YT (NK cell), HL-60 (monocytic), and DB (B cell), in addition to a murine lymphoma cell line (YAC-1), were cultured in RPMI 1640 supplemented with 10% fetal calf serum (Hyclone, Logan, Utah), 2 mM L-glutamine, 100 Units/ml of penicillin and steptomycin, 1 mM sodium pyruvate, and 0.1 mM non-essential amino acids (Gibco BRL). A lymphokine-activated killer cell (LAK) culture was obtained by isolating peripheral blood mononuclear cells (PBMC) from 60 ml of venous blood from a healthy donor by Ficol-Paque centrifugation (Pharmacia, Pescataway, N.J.). The cells were grown in the above media supplemented with 1000 Units/ml of human rIL-2 for three days. The non-adherent cells were removed and the culture was continued in 500 Units/ml of human rIL-2 and conditioned media until day 10, when RNA was extracted. All cell lines were grown to one million per ml and split 1:2 24 h before RNA isolation.

EXAMPLE 3 - RNA and DNA Blot Analysis

Total RNA was isolated with the RNAstate 60 reagent according to the manufacturer's protocol (Teltest Inc., Friendswood, Tex.), divided into 20 µg aliquots, and stored in 70% EtOH at –80° C until used. One percent agarose gels for northern analysis were stained with ethidium

bromide after electrophoresis to insure equal loading by comparison of rRNA. Northern blots were probed with 25 ng of the full-length cDNA labeled with α^{32}P dCTP (Feinberg and Vogelstein 1983; Sambrook et al. 1989). The first blot consisted of 20 μg of total RNA from human monocytic, T, B, and NK cell lines (HL-60, Jurkat, DB, and YT, respectively), a mouse cell line (YAC-1), and LAC and PBMC cells from a healthy donor immobilized on Hybond nylon (Amersham, Arlington Heights, Ill.). Prehybridization and hybridizations were performed according to the instructions of Amersham for the Hybond nylon membrane at 65° C. The second membrane was purchased from Clontech (Palo Alto, Calif.) and contained mRNA samples from human spleen, lymph node, thymus, peripheral blood leukocytes, bone marrow, and fetal liver (human immune system multiple tissue northern blot II). It was hybridized according to the manufacturer's instructions with the included Express-Hyb Hybridization solution at 65° C. Blots were exposed to Hyperfilm (Amersham). The membrane was subsequently stripped and reprobed for β actin to insure equal loading.

Genomic DNA was isolated from human liver according to standard protocol (Sambrook et al. 1989). For DNA blot analysis, human genomic DNA samples (20 μg each) were digested with various restriction enzymes (BamHI, EcoRI, HindIII, and XbaI) and separated on 0.8% agarose gel by electrophoresis. The DNA was transferred to Hybond nylon membrane under alkaline conditions (0.4 N NaOH), and fixed by UV cross linking. The membrane was prehybridized for 2 h at 65° C in hybridization buffer (1 mM ethylenediaminetetraacetate (EDTA), 0.5 M sodium phosphate, pH 7.2, 7% sodium dodecyl sulfate (SDS), 100 μg/ml ssDNA). The probe (50 ng of the full-length cDNA labeled with α^{32}P dCTP) was added to the same buffer and hybridization was continued for 18 h at 65° C (Feinberg and Vogelstein 1983; Sambrook et al. 1989). The membrane was washed with a buffer containing 40 mM sodium phosphate, pH 7.2, 1% SDS at 65° C for 1 h. The membrane was exposed to Hyperfilm for one day (Amersham) and developed.

PAC identification

A gridded human PAC library (RPCI1) was hybridized for 20 h with 40 ng of LLT1 (full-length cDNA labeled with $\alpha^{32}P$) (Feinberg and Vogelstein) 1983; Ioannou et al. 1994; Sambrook et al. 1989). The membranes were washed twice with 2 X standard sodium citrate, 0.1% SDS at 65° C for 20 min and exposed to X-ray film at -70° C for 24 h. Positive clones were provided by the Human Genome Mapping Project resource center. DNA was extracted using a Qiagen kit (Crawley, UK) according to standard protocols. PAC DNA (200 ng) was digested to excise the insert and separated on a pulse field gel with ramped switch times from 1 to 13 s at 200 V for 16 h.

PAX polymerase chain reaction and sequencing

PCR was performed in the presence of 2.5 mM $MgCl_2$ using primers designed to the cDNA sequence (RBC151, RBC130, RBC136, and RBC141; Table 1). PAC DNA was treated for 10 s at 94° C, followed by 30 cycles at 94° C for 10 s, 55° C for 10 s, and 72° C for 2 min with a 10 min final extension at 72° C. The PCR products were purified using a PCR purification kit from Qiagen and sequenced on an ABI377 automated sequencer as described previously (Wilson et al. 1997)

In order to understand the mechanism by which NK cells recognize and kill target cells, the present inventors searched the EST database with a consensus sequence of human (CD69, CD94, and NKG2s) and mouse (Ly-49s) C-type lectin receptors (Boguski et al. 1993; Boguski 1995). Several overlapping clones were identified and PCR primers were designed and used in PCR to yield a 350 bp fragment within the C-type lectin CRD. The primers used for PCR amplifications are given in Table 1. A human NK cell cDNA library was screened with the PCR fragment and a positive clone (Y9A2) was selected for further analysis. The clone contained a cDNA insert of 850 bp with an open reading frame predicting a polypeptide of 191 amino acid residues with a type II receptor structure (Genbank accession number AF133299). The predicted protein sequence had a single transmembrane domain of 29 amino acid residues (FIG. 1A, B)

and an intracellular domain of 30 amino acid residues. Additionally, it had an extracellular lectin-like domain of 132 amino acid residues which contained two putative N-linked glycosylation sites (FIG. 1A).

The predicted protein sequence of LLT1 has an extracellular domain with some similarity to the C-type lectin-like domains shared with other NK cell receptors (FIG. 2). It has the highest similarities to AICL and CD69 of 59 and 56%, respectively (Hamann et al. 1993, 1997; Lopez-Cabrera et al. 1993; Ziegler et al. 1993). Representative similarities to other NK cell receptors belonging to the C-type lectin superfamily are 53, 51, and 41% to NKG2-D, CD94, and Ly-49D, respectively (Chang et al. 1995; Houchins et al. 1991; Weis et al. 1998; Wong et al. 1991).

Analysis of the sequences of LLT1 clones from another NK cell cDNA library made from pooled NK cells (NKTRP) revealed no differences, indicating that the gene is not highly polymorphic. Sequence data available in the EST database did not show variation beyond what was expected for single-pass sequences.

EXAMPLE 4 - Expression of LLT1 in different tissues and cells

The expression of LLT1 transcripts in various cell lines and different human tissues was analyzed by northern blotting of total RNA or poly(A)$^+$ RNA. The full-length cDNA hybridized to transcripts of approximately 5, 3.5, 2, and 0.9 kilobases (kb) in total RNA from a human NK cell line (YT) and hybridized weakly to transcripts of similar sizes from human T cell (Jurkat), B cell (DB), or monocytic (HL-60) tumor cell lines. Hybridization signals for the same size transcripts were strong in donor samples from a LAK culture and PBMC except for the 900 bp transcript (FIG. 3A). Tissue distribution of LLT1 showed that human peripheral blood leukocytes, lymph node, thymus, and spleen expressed transcripts of the same relative sizes as the YT cell line with the exception of the 900 bp transcript (FIG. 3B). No hybridizing transcripts were detected in mRNA from fetal liver or bone marrow. LLT1 may be expressed only in the later stages of NK cell differentiation, similar to Ly49 expression.

Southern analysis of human genomic DNA

Several lectin-like receptors expressed on NK cells belong to multigene families (Lanier 1998; Long and Wagtmann 1997; Weis et al. 1998). Southern blot analysis of human genomic DNA was carried out to explore this possibility for LLT1. Genomic DNA was isolated from human liver and digested with four different restriction enzymes (BamHI, EcoRI, HindIII, and XbaI), separated on an agarose gel and transferred to a nylon membrane. The full-length LLT1 cDNA hybridized to several restriction fragments (FIG. 4). The strongly hybridizing restriction fragments identified in FIG. 4 ranged from 22 to 35 kb for the different digestions in addition to several weakly hybridizing bands.

EXAMPLE 5 - Chromosomal Localization

Due to the sequence similarity of LLT1 clone to AICL and CD69, LLT1 may be localized in the NK gene complex on Chr 12. Therefore, a PAC library containing the NK gene complex was screened. Two PACs were isolated from the human RCP1 library using a probe for LLT1. The inserts of PAC NKCP4 and NKCP5 were sized on a pulsed field gel to 110 and 160 kb, respectively.

LLT1 is located in the human NK gene complex within 100 kb of the CD69 gene. PCT products of approximately 0.9 kb (RBC150/RBC130) as expected for the CD69 gene and 1.8 kb (RBC136/RBC141) for the LLT1 gene were obtained with both PAC DNAs as templates. The PCR products were sequenced. The LLT1-specific PCR product sequence revealed amplification of an intron. The exon sequence showed 100% identity to the cDNA of LLT1. The presence of an intron sequence is consistent with this being the authentic LLT1 gene, and not a process pseudogene. Consistently, all CD69 sequences were observed to have three nucleotide exchanges (out of 796 bp) in comparison with the published CD69 sequence (GenBank accession number Z30428). This may be due to polymorphism in the untranslated 3' end of the gene.

The observation of multiple bands in northern analysis implies the existence of highly related transcripts or splice variants of LLT1 (FIG. 3). Both LLT1 and CD69 are expressed in lymphocytes, at a high level in NK cells and less in T and B cells (FIG. 3; Hamann et al 1993). LLT1 may be inducible on T and B cells. The restricted expression of LLT1 to tissues representing the later stages of NK cell differentiation implicates it as a receptor involved in immune response rather than development (FIG. 3B). The Southern blot showed a simple pattern indicting a single gene or a small number of genes. A somewhat similar pattern has been reported for CD69 (Santis et al. 1994) and AICL (Hamann et al. 1997). The CD69 gene is localized to approximately 20 kb. Taken together, LLT1 is likely a single gene.

The LLT1 gene was localized to within 100 kb of the CD69 gene. The AICL gene has been previously localized to 0.3 cM proximal to the CD69 gene (Hamann et al. 1997). The genes might be derived from the duplications of a common ancestral gene in view of the genes and the sequence similarities between he AICL, CD69, and LLT1 cDNAs.

Sequence analysis and chromosomal localization classify LLT1 as a new member of the NK cell receptors located in the human NK gene complex. Sequence similarity to CD69 and AICL suggest that LLT1 may have a comparable role in the immune system. Antibodies are being generated which will permit the functional role of LLT to be discerned in human lymphocytes.

EXAMPLE 6 - Characterization of a Novel Lectin-like Transcript (LLT2) Expressed in Humans

Natural killer (NK) cells are lymphocytes that spontaneously detect and kill cancerous and virally infected cells through receptors that transduce either activating or inhibiting signals. Many NK cell receptors belong to the C-type lectin superfamily and include CD69, Ly-49, and the NKG2/CD94 heterodimer. A lectin-like transcript (LLT1) expressed on human NK cells that maps to the NK gene complex on chromosome 12 has previously been identified by the present inventors. A second human lectin-like transcript (LLT2) has also been characterized

molecularly. The cDNA encodes a predicted protein of 242 amino acid residues with a transmembrane domain near the N-terminus and an extracellular domain of 199 amino acid residues with homology to the carbohydrate recognition domain of C-type lectins. The predicted protein of LLT2 show 46, 49, and 51% similarity to NKG2-D, CD94, and LLT1, respectively. The predicted protein does not contain intracellular ITIM motifs suggesting that LLT2 may be involved in mediating activation signals.

EXAMPLE 7 – Preparation of Recombinant LLT1 Peptide Using the LLT1 cDNA in a Bacterial Expression System

The cDNA for LLT1 will be digested with restriction enzymes and cloned into a suitable protein expression vector. The expression construct plasmid will be purified and transected into *E. coli* by standard procedures. Expression of the recombinant peptide was performed following the expression vector supplier's recommended protocols LLT1 expression cultures would be expected to yield several milligrams of protein per liter on average. The recombinant polypeptide of 29-30 kDa will be purified by standard procedures such as polyacrylamide gel electrophoresis, isoelectric focusing, and FPLC over cation, anion, gel filtration, and/or Hydroxyapatite columns.

The purified protein will then used to generate polyclonal antibodies. New Zealand White rabbits will be immunized with 1-10 milligrams of recombinant purified LLT1 mixed with Freund's adjuvant following standard protocols. After multiple injections over several weeks, the rabbits whose sera are positive for LLT1 antibodies will be sacrificed and their sera stored at –20 degrees C.

The purified protein will also be used to generate monoclonal antibodies. Mice (e.g. BALB/C) will be immunized with recombinant purified LLT1 mixed with Freund's adjuvant following standard protocols. After multiple injections over time, the mice will be sacrificed and fusion hybridoma cells generated using spleen derived B-cells by following standard protocols. Hybridoma cell lines will then be isolated by serial dilution cloning and lines positive for LLT1 antibodies will be identified using standard protocols.

EXAMPLE 8 – Fusion Protein LLS1

Bacterial protein is a fusion protein used in AB products β-gal (fan bacterial) = LLT1 aa

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the composition, methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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